REMARKS

I. Status Summary

Claims 7, 11, 13, 59, and 101-103 are pending and have been examined in the instant application and have been examined by the U.S. Patent and Trademark Office (hereinafter "the Patent Office").

Claims 7, 11, 13, 59, and 101-103 have been rejected under 35 U.S.C. § 112, first paragraph, upon the contention that the specification, while enabling for SEQ ID NO: 15, is non-enabling for a polypeptide encoded by a nucleic acid sequence having 90% or more sequence identity to the nucleotide sequence of SEQ ID NO: 15.

Claims 7 and 104 have been amended. Claim 7, subsection (b), has been amended to recite that the isolated and purified nucleic acid molecule encoding the biologically active KCC3a potassium-chloride cotransporter polypeptide comprises a nucleic acid sequence having 90% or greater sequence identity to SEQ ID NO 15 and encodes a biologically active KCC3a polypeptide comprising an amino acid sequence at least 95% identical to SEQ ID NO: 16. Support for the amendment to claim 7 can be found in throughout the specification as filed, including particularly in the Sequence Listing (minimum 90% identity or 95% identity between mouse and human KCC3a, and between different KCC3a nucleic acids and polypeptides, respectively). Claim 104 has been amended to correct a typographical error. No new matter has been added with the amendments to claims 7 and 104.

New claims 105-110 have been added. Support for the new claims can be found throughout the specification as filed, including in the claims as originally filed. Additional support for new claim 105 can be found in the Sequence Listing (minimum 95% identity between mouse and human KCC3a, and between different KCC3a polypeptides). Additional support for new claims 106-110 can be found in the original claims, including particularly claim 11 (nucleic acid under the control of a promoter), claim 13 (recombinant host cell comprising the nucleic acid), claim 12 (recombinant vector), and in the specification as filed, including particularly at page 51, lines 16-19 (recombinant expression vector), in the Sequence Listing (nucleotides 165-434 of SEQ ID NO: 15

encode amino acids 1-90 of SEQ ID NO: 16), and at page 64, lines 17-23 (100 nucleotide contiguous stretch of SEQ ID NO: 15). Accordingly, no new matter has been added by virtue of the new claims.

Reconsideration of the application based on the remarks set forth herein below is respectfully requested.

II. Response to the Rejections Under 35 U.S.C. §112, First Paragraph

Claims 7, 11, 13, 59, and 101-103 have been rejected under 35 U.S.C. § 112, first paragraph, upon the contention that the specification, while enabling for SEQ ID NO: 15, is non-enabling for a polypeptide encoded by a nucleic acid sequence having 90% or more sequence identity to the nucleotide sequence of SEQ ID NO: 15. According to the Patent Office, the claims recite a nucleic acid having 90% sequence identity to the KCC3 nucleotide of SEQ ID NO: 15. The claims are also asserted to embrace variants of SEQ ID NO: 16, such as a peptide that has potassium-chloride cotransporter activity, or those that are immunologically cross-reactive, or those encoded by a nucleic acid that hybridizes to the first 434 nucleotides of SEQ ID NO: 15. The Patent Office further asserts that the specification does not enable the various protein forms of the KCC3 transporter, wherein the nucleic acid sequence is 90% identical to SEQ ID NO: 15, with the assurance that enabled proteins that are functionally equivalent to SEQ ID NO: 16 can be made without undue experimentation and with the assurance that they would have the desired properties of the claimed KCC3A transporter.

After careful consideration of the rejections and the Patent Office's bases therefor, applicants respectfully traverse the rejections and submit the following remarks.

Initially, applicants respectfully submit that several of the above referenced assertions are inaccurate with respect to the claims as amended in the previous Amendment, and thus it appears that the Patent Office is basing at least part of the instant rejection on a version of the claims that is not longer applicable. For example,

the Patent Office asserts the claims recite nucleic acids that encode polypeptides that are immunologically cross reactive with SEQ ID NO: 16. Applicants respectfully submit that the subsection that recited immunological cross-reactivity was deleted from claim 7 in the previous Amendment.

Additionally, applicants respectfully submit that the claims do not recite simply that the polypeptides are encoded by a nucleic acid that hybridizes to the first 434 nucleotides of SEQ ID NO: 15. This element appears in claim 1, which has been withdrawn from consideration.

Therefore, applicants respectfully submit that at least part of the instant rejection of claims 7, 11, 13, 59, and 101-103 appears to be based on an understanding of the breadth of the subject matter of the claims that is inaccurate, and thus applicants respectfully request that these assertions be withdrawn by the Patent Office.

Continuing with the instant rejection, it appears that the instant rejection is based on the sole assertion that the specification does not enable nucleic acid molecules at least 90% identical to SEQ ID NO: 15 that encode a biologically active KCC3a potassium-chloride cotransporter. In support of this rejection, the Patent Office asserts the following:

1. that the claims refer to any polynucleotide or polypeptide that is "at least 90% identical" to that of SEQ ID NO: 15 or 16 "without knowledge of the polynucleotides or polypeptides that would fall within this range";
2. that there is no discussion of working examples;
3. that there is no discussion of what residues are necessary to maintain the functional characteristics of the claimed polynucleotides;
4. that the specification teaches that the KCC transporters each show different selectivity, sensitivity, and activity;
5. that antibodies raised against KCC do not cross react with the other KCC transporters;

6. that there is nothing unusual in the exons from different transporters within a family bearing a superficial resemblance to one another, but that one cannot infer that they are functionally equivalent because of the resemblance; and
7. that the instant claims suggest altering as much as 10% of the polypeptide disclosed in SEQ ID NO: 16, but that prior art teaches that transporters with high homology do not always share a specific and substantial functional attribute or utility despite structural similarity.

Turning first to Assertion Nos. 4-6, applicants respectfully submit that these assertions do not support the instant rejection. Applicants respectfully submit that even assuming *arguendo* that these assertions are accurate, the differences between the selectivities, sensitivities, and activities (Assertion No. 4) of different KCC transporters are not relevant to the subject matter of the instant claims, which recite KCC3a transporters. As indicated in response to the previous Official Action, KCC3a transporters are all transcribed from the KCC3 locus, whereas KCC2 and KCC4 are transcribed from entirely different loci. The human KCC2 locus is on chromosome 20, the human KCC3 locus is on chromosome 15, and the human KCC4 locus is on chromosome 5. Accordingly, applicants respectfully submit that whether these various KCC polypeptides have the same or different biological activities or arose from a common precursor gene has no bearing on the instant claims.

Additionally, the Patent Office's assertion that antibodies that bind to KCC3 do not bind to other KCCs (Assertion No. 5) also fails to support the instant rejection. Particularly, the Patent Office asserts on page 4 of the Official Action that this lack of cross-reactivity supports the contention that the KCC transporters are separate inventions. Applicants respectfully submit, however, that even assuming *arguendo* that this assertion is accurate with respect to a Restriction/Election Requirement, it does not support the instant rejection because as one of ordinary skill in the art would understand upon consideration of the instant specification, the antibodies employed to generate the data presented in Figure 27D were raised against a region of human KCC3 polypeptide that is encoded by exon 3, which is clearly not present in hKCC 2, hKCC4, or *Xenopus*

KCC. Interestingly, the sequence to which the antibody was raised is present in all human and mouse KCC3s, including KCC3a, KCC3a-X2, and KCC3b. Applicants respectfully note, therefore, that if the Patent Office's assertions that lack of cross-reactivity implies separate inventions, the existence of cross-reactivity of this antibody with these polypeptides suggests that SEQ ID NOs. 3-10, 15, and 16 should be examined together.

And finally, applicants respectfully submit that contrary to Assertion No. 6, there is more than a "superficial" resemblance among the coding sequences of human and mouse KCCs. A summary of the percent homologies among these open reading frames is presented in the following Table.

	hKCC3a-X2 Seq Id No. 3	mKCC3a-X2 Seq Id No. 5	mKCC3a Seq Id No: 7	mKCC3b Seq Id No. 9	hKCC3a Seq Id No. 15
hKCC3a-X2 Seq Id No. 3	-	3077/3405 (90%)	3080/3405 (90%)	2835/3132 (90%)	3404/3408 (99%)
mKCC3a-X2 Seq Id No. 5	-	-	3071/3395 (90%)	3131/3135 (99%)	3071/3395 (90%)
mKCC3a Seq Id No: 7	-	-	-	3175/3180 (99%)	3121/3450 (90%)
mKCC3b Seq Id No. 9	-	-	-	-	2879/3180 (90%)
hKCC3a Seq Id No. 15	-	-	-	-	-

Thus, as between SEQ ID NO: 15 (hKCC3a) and the other sequences, there is far more than just a mere resemblance in the sequences.

Accordingly, applicants respectfully submit that the Patent Office's assertions concerning evolutionary considerations of gene and/or exon duplication has no bearing on the instant claims, which recite KCC3a nucleic acids, polypeptides, and derivatives thereof, and thus does not support the instant rejection.

Turning now to Assertion Nos. 1 and 2, applicants respectfully submit that contrary to these assertions, the specification in fact discloses four (4) different nucleic acid sequences and four (4) different polypeptide sequences that all fall within the 90% sequence identity element recited in the claims. These sequences are SEQ ID NOs. 3-8, 15, and 16, which correspond to human and mouse versions of KCC3a and KCC3a-X2. The maximum sequence divergence between any of these sequences according to the BLAST algorithm available from the website of the National Center for Biotechnology Information (NCBI) is 10% at the nucleic acid sequence level and 4% at the amino acid sequence level.

Therefore, contrary to the Patent Office's assertion, the specification does indeed teach several potassium-chloride cotransporters that fall within the scope of the claims. Thus, applicants respectfully submit that these sequences represent "working examples" that inform one of ordinary skill in the art concerning the nature of the presently disclosed subject matter.

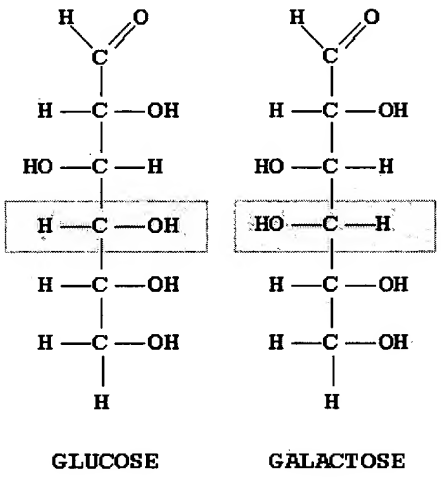
With respect to Assertion Nos. 3 and 7, applicants respectfully submit that these assertions also do not support the instant rejection. First, applicants respectfully submit that the Patent Office's reliance on Oelmann *et al.*, Bisson *et al.*, and Liang *et al.* in Assertion No. 7 fails to support the instant rejection because the Patent Office is overestimating the impact of these publications on the predictability of the art with respect to creating amino acid changes in proteins, and underestimating the direction provided by the instant specification to guide the skilled artisan in performing amino acid changes.

To elaborate, Oelmann *et al.* teaches that changes in three different amino acids at positions that are highly conserved in all known mammalian nucleotide-sugar transporters resulted in inactivation of the transporters. Applicants respectfully submit that this disclosure does not support the instant rejection because one of ordinary skill in the art would reasonably have believed that amino acid residues that are highly conserved throughout mammalian evolution would be poor candidates for substitution in designing polypeptide variants. Thus, applicants respectfully submit that one of

ordinary skill in the art would have been disinclined to attempt the amino acid changes disclosed in Oelmann *et al.* when the goal is to maintain biological activity as recited in the instant claims.

Turning now to Bisson *et al.*, applicants respectfully submit that the degree of non-homology between the galactose and glucose transporters disclosed therein (*i.e.*, 16.3%) is almost twice that recited in the instant claims. Thus, the applicability of Bisson *et al.* to the instant claims is dubious at best.

Furthermore, applicants respectfully submit that the considerable degree of non-homology disclosed in Bisson *et al.* only resulted in a change in substrate specificity related to the relative positions of a single OH group as depicted below.



Accordingly, applicants respectfully submit that Bisson *et al.* does not support the instant rejection as applied to potassium-chloride cotransporters, which cotransport ions. In fact, applicants respectfully submit that when taken in its entirety, Bisson *et al.* can be interpreted to teach that even a large degree of amino acid substitution can result in a polypeptide that has at most a very subtle change in biological activity.

And finally, applicants respectfully submit that Liang *et al.* also fails to support the instant rejection. Applicants respectfully submit that Liang *et al.* *might* stand for the proposition that amino acid changes can result in new activities for polypeptides as represented by the ability of single mutations to confer on mutant hexose transporters

the ability to transport potassium. However, applicants respectfully submit that the nature of the experiments performed in Liang *et al.* differs from a strategy designed to produce variant KCC3a polypeptides that retain potassium-chloride cotransporter activity. Particularly, the mutations disclosed in Liang *et al.* were identified under selective pressure in order to identify extragenic suppressors of potassium transporter double mutants.

Stated another way, Liang *et al.* did not generate variant potassium transporters and show that mutations could alter potassium transport, but rather placed cells under selective pressure in an attempt to identify gain-of-function mutations in unrelated proteins. Applicants respectfully note that several of these mutants still retained the ability to transport glucose, and thus for the purposes of the instant claims, these embodiments are properly considered gain-of-function mutants vis-à-vis potassium transport and not loss-of-function mutants vis-à-vis hexose transport. Thus, each of these embodiments actually supports applicants' contentions that mutations in polypeptides can be accommodated without loss of function.

Furthermore, even assuming *arguendo* that the Liang *et al.* reference teaches that certain mutations can give rise to altered biological activities, it does not teach as broadly as the Patent Office appears to assert that generally, the few amino acid substitutions that do change substrate specificity "dramatically" lead to a level of unpredictability that would necessarily require undue experimentation. In fact, applicants concede that certain types of mutations would be expected to destroy the function of any given polypeptide. For example, a mutation that introduces a stop codon in the mRNA of the polypeptide would be expected to destroy the biological activity of the polypeptide.

Applicants respectfully submit, however, that in order to satisfy the enablement requirement of 35 U.S.C. § 112, first paragraph, it is not necessary that every embodiment that literally falls within the scope of the claim be operative. Rather, the Court of Appeals for the Federal Circuit (CAFC) stated in *Atlas Powder Co. v. E.I. DuPont De Nemours & Co.* (750 F.2d 1569, 1576-1577, 224 USPQ 409, 414 (Fed. Cir.

1984)) that "It is not a function of the claims to specifically exclude ... possible inoperative substances...." (*citing In re Dinh-Nguyen,* 492 F.2d 856, 858-59, 181 USPQ 46, 48 (CCPA 1974; emphasis omitted); *In re Geerdes,* 491 F.2d 1260, 1265, 180 USPQ 789, 793 (CCPA 1974); and *In re Anderson,* 471 F.2d 1237, 1242, 176 USPQ 331, 334-35 (CCPA 1973)). In *Atlas Powder*, the CAFC also held that unless the number of inoperative combinations becomes "significant" to force one of skill in the art to experiment unduly, the claims would be valid – *i.e.*, would satisfy the requirements of 35 U.S.C. § 112, first paragraph.

Applicants further respectfully submit that after consideration of the instant specification, one of ordinary skill in the art would be able to choose particular amino acid substitutions that would be expected to be tolerated. For example, given the disclosure of several different KCCs that could be used as a guide for possible alterable positions, applicants respectfully submit that one of ordinary skill in the art could create such mutations by routine (*i.e.*, not undue) experimentation, and could also test the same using the disclosed assays.

Therefore, since there is no requirement that the specification include an explicit discussion of amino acids that are necessary to maintain the functional characteristics of the claimed polynucleotides and polypeptides (Assertion No. 3), and further because the instant specification must be considered in light of the knowledge and abilities of the skilled artisan, applicants respectfully submit that it is clear that the specification's disclosure of multiple KCC3a sequences informs one of ordinary skill in the art as to what amino acids might be required and what amino acids might tolerate changes. Furthermore, applicants respectfully submit that after review of the instant specification, one of ordinary skill in the art would understand what amino acid changes might be expected to be tolerated in SEQ ID NO: 16 without destroying the biological activity of the encoded polypeptide. Applicants respectfully submit that the specification discloses 7 different KCC polypeptide sequences and the nucleic acid sequences encoding the same. One of ordinary skill in the art could easily perform an alignment of the amino acid sequences in order to determine regions of the KCC3a polypeptides that would be

good candidates for amino acid substitutions. Applicants respectfully submit that this does not constitute undue experimentation.

Such an alignment is presented in **EXHIBIT A** submitted herewith. It can be seen that among mouse and human versions of KCC2, KCC3a, KCC3a-X2, KCC3b, and KCC4, there are regions of conservation and other regions of variability. Applicants respectfully submit that even between human KCC3a (SEQ ID NO: 16) and mouse KCC3a (SEQ ID NO: SEQ ID NO: 8), there are 22 amino acid changes, and further that one of ordinary skill in the art could routinely produce other amino acid substitutions at these positions and test the resultant polypeptides for potassium-chloride cotransporter activity. This does not even take into account the additional fact that the level of skill in the art of molecular biology is extremely high, and one of ordinary skill in this technology area could routinely generate various conservative substitutions in many of the other residues and still retain potassium-chloride cotransporter activity. Once these polypeptides were confirmed as biologically active, which applicants respectfully submit that could also be accomplished routinely via the assays disclosed in the specification, one of ordinary skill in the art would then be able to generate a large number of nucleic acid molecules that are at least 90% identical to SEQ ID NO: 15 and that encode the biologically active polypeptides.

Furthermore, applicants respectfully submit that claim 7 has been amended to recite *inter alia* that the nucleic acid molecules, while they can diverge from SEQ ID NO: 15 by as much as 10%, still encode a polypeptide that is at least 95% identical to SEQ ID NO: 16 and has potassium-chloride cotransporter activity. As such, applicants respectfully submit that the Patent Office's assertion that claim 7 encompasses amino acid sequences that can diverge from SEQ ID NO: 16 by as much as 10% is no longer applicable. Applicants further respectfully submit that the Patent Office has already taken the position in Example 14 of the Synopsis of Application of Written Description Guidelines that "procedures for making variants of [an amino acid sequence] which have 95% identity to [the amino acid sequence] and retain its activity are conventional in the art". As such, applicants respectfully submit that the amendments presented herein

are consistent with the stance taken by the Patent Office in Example 14 of the Synopsis of Application of Written Description Guidelines that generating polypeptides that are at least 95% amino acid identical to a known sequence of a polypeptide plus retain the function of the known polypeptide are "conventional in the art" (*i.e.*, are enabled).

Applicants further respectfully submit that for each and every amino acid sequence so generated, one of ordinary skill in the art can routinely generate each and every nucleic acid sequence that encodes that amino acid sequence. It is noted, also, that many of these nucleic acid sequences would diverge from SEQ ID NO: 15 by more than 10%, even in the open reading frame, due to the redundancy of the genetic code. Therefore, applicants respectfully submit that one of ordinary skill in the art of molecular biology could routinely make variants of SEQ ID NO: 16 that are at least 95% identical to SEQ ID NO: 16 and retain its activity (*i.e.*, potassium-chloride cotransporter activity) and that are encoded by nucleic acid molecules at least 90% identical to SEQ ID NO: 15 as recited in claim 7(b) using conventional techniques.

Summarily, applicants respectfully submit that the Patent Office has provided no support for the contention that after consideration of the instant specification, one of ordinary skill in the art would be unable to design nucleic acid molecules that are at least 90% identical to SEQ ID NO: 15 and encode biologically active KCC3a transporters that have amino acid sequences at least 95% identical to SEQ ID NO: 16. Applicants respectfully submit that (a) the specification provides examples of embodiments of the nucleic acids and polypeptides that fall within the scope of the claims; (b) the KCCs as a family have been well characterized; (c) the level of skill of one of ordinary skill in the art is extremely high; (d) the level of predictability in the art has been underestimated by the Patent Office, particularly in view of the teachings of the instant specification when augmented by the knowledge one of ordinary skill in the art would have had of the KCC3 family as of the filing date of the instant application; and (e) the quantity of experimentation required to make and use the invention based on the content of the disclosure in combination with the knowledge of the skilled artisan would be simply routine.

Therefore, applicants respectfully submit that proper consideration of the so-called *Wands* Factors leads to the conclusion that the instant claims comply with the requirements of 35 U.S.C. § 112, first paragraph. Applicants have provided extensive support for the contention that one of ordinary skill in the art could easily design additional nucleic acid molecules that are considerably more divergent than 10% from SEQ ID NO: 15 and yet would be expected to be biologically active. Therefore, even if some of the nucleic acids that are at least 90% identical to SEQ ID NO: 15 would represent non-working embodiments, applicants respectfully submit that this does not support a rejection of claims 7, 11, 13, 59, and 101-103 under the enablement provision of 35 U.S.C. § 112, first paragraph.

Accordingly, applicants respectfully submit that the rejection of claims 7, 11, 13, 59, and 101-103 under 35 U.S.C. § 112, first paragraph, has been addressed, and that the claims are in condition for allowance. Applicants respectfully request a Notice of Allowance to that effect.

Discussion of the New Claims

New claims 105-110 have been added. Support for the new claims can be found throughout the specification as filed, including in the claims as originally filed. Additional support for new claim 105 can be found in the Sequence Listing (minimum 95% identity between mouse and human KCC3a, and between different KCC3a polypeptides). Additional support for new claims 106-110 can be found in the original claims, including particularly claim 11 (nucleic acid under the control of a promoter), claim 13 (recombinant host cell comprising the nucleic acid), claim 12 (recombinant vector), and in the specification as filed, including particularly at page 51, lines 16-19 (recombinant expression vector), in the Sequence Listing (nucleotides 165-434 of SEQ ID NO: 15 encode amino acids 1-90 of SEQ ID NO: 16), and at page 64, lines 17-23 (100 nucleotide contiguous stretch of SEQ ID NO: 15). Accordingly, no new matter has been added by virtue of the new claims.

New claims 105-110 are believed to be in compliance with the enablement provision of 35 U.S.C. § 112, first paragraph, for the reasons set forth hereinabove with respect to claims 7, 11, 13, 59, and 101-103. Accordingly, applicants respectfully submit that claims 105-110 are also believed to be in condition for allowance, and respectfully solicit a Notice of Allowance to that effect.

CONCLUSIONS

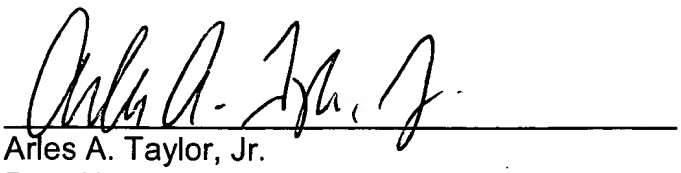
In light of the above amendments and remarks, applicants submit that the subject patent application is in condition for allowance and courteously solicit a Notice of Allowance.

If any small matter should remain outstanding after the Patent Examiner has had an opportunity to review the above Remarks, the Patent Examiner is respectfully requested to telephone the undersigned patent attorney in order to resolve these matters and avoid the issuance of another Official Action.

Deposit Account

The Commissioner is hereby authorized to charge any deficiencies of payment associated with the filing of this correspondence to Deposit Account No. **50-0426**.

Respectfully submitted,
JENKINS, WILSON, TAYLOR & HUNT, P.A.

Date: July 24, 2006 By: 

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1242/26/2 AAT/CPP/sla

EXHIBIT A

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mKCC3a-X2       MHPPEATTKMSSVRFMVTPTKIDDIPGLSDTSPDLSSRSSSRVRFSSRESVPETSRSEPM
mKCC3a          MHPPEATTKMSSVRFMVTPTKIDDIPGLSDTSPDLSSRSSSRVRFSSRESVPETSRSEPM
hKCC3a-X2       MHPPETTTKMASVRFMVTPTKIDDIPGLSDTSPDXSSRSSSRVRFSSRESVPETSRSEPM
hKCC3a          MHPPETTTKMASVRFMVTPTKIDDIPGLSDTSPDXSSRSSSRVRFSSRESVPETSRSEPM
mKCC3b          -----------MPHFTVTKVEDPEEGAAGPLSPEPSS-AEVKARIQDPQ-EPDPSQNS--
hKCC4           ---------------MPTNFTVVPVEAHADGGGDETAERTEAPGTPEGPEPERPSPGD--
mKCC4           ---------------MPTNFTVVPVEARADGAGDEAAERTEEPESPESVDQTSPTPGD--
hKCC2           ---------------MPNNLTDC-----EDGDGG------------------ANPGD--
                                                                  .. ..

mKCC3a-X2       SELSGATTSLATDPSSDRTSNPQDVTED---------------DGHKKARNAYXNNSNYE
mKCC3a          SELSGATTSLATDPSSDRTSNPQDVTEDPSQNSITGEHSQLLDDGHKKARNAYLNNSNYE
hKCC3a-X2       SEMSGATTSLATDPPSDRTSHPQDVIE---------------DDGHKKARNAYLNNSNYE
hKCC3a          SEMSGATTSLATDPPSDRTSHPQDVIEDLSQNSITGEHSQLLDDGHKKARNAYLNNSNYE
mKCC3b          --ITGEHSQLLDD------------------------------GHKKARNAYXNNSNYE
hKCC4           ---------------------------------------------GNPRENSPFLNNVEVE
mKCC4           ---------------------------------------------GNPRENSPFINNVEVE
hKCC2           ---------------------------------------------GNPKESSPFINSTDTE
                                                           *: :  ..: *. : *.

mKCC3a-X2       EGDEYFDKNLALFEEEMDK---VSSLLNRMANYTNLTQGAKEHEEAENITEGKKKPTKSP
mKCC3a          EGDEYFDKNLALFEEEMDK---VSSLLNRMANYTNLTQGAKEHEEAENITEGKKKPTKSP
hKCC3a-X2       EGDEYFDKNLALFEEEMDK---VSSLLNRMANYTNLTQGAKEHEEAENITEGKKKPTKTP
hKCC3a          EGDEYFDKNLALFEEEMDK---VSSLLNRMANYTNLTQGAKEHEEAENITEGKKKPTKTP
mKCC3b          EGDEYFDKNLALFEEEMDK---VSSLLNRMANYTNLTQGAKEHEEAENITEGKKKPTKSP
hKCC4           QESFFEGKNMALFEEEMDSNPMVSSLLNKLANYTNLSQGVVEHEEDEES---RRREAKAP
mKCC4           RESYFEGKNMAXFEEEMDSNPMVSSLLNKLANYTNLSQGVVEHEEDEDS---RRREVKAP
hKCC2           KGKEYDGKNMALFEEEMDTSPMVSSLLSGLANYTNLPQGSREHEEAENNEGGKKKPVQAP
                . . : .**:* ******.   *****. :******.**  **** *:    ::: .::*

mKCC3a-X2       QMGTFMGVYLPCLQNIFGVILFLRLTWVVGTAGILQAFAIVLICCCCTMLTAISMSAIAT
mKCC3a          QMGTFMGVYLPCLQNIFGVILFLRLTWVVGTAGILQAFAIVLICCCCTMLTAISMSAIAT
hKCC3a-X2       QMGTFMGVYLPCLQNIFGVILFLRLTWVVGTAGVLQAFAIVLICCCCTMLTAISMSAIAT
hKCC3a          QMGTFMGVYLPCLQNIFGVILFLRLTWVVGTAGVLQAFAIVLICCCCTMLTAISMSAIAT
mKCC3b          QMGTFMGVYLPCLQNIFGVILFLRLTWVVGTAGILQAFAIVLICCCCTMLTAISMSAIAT
hKCC4           RMGTFIGVYLPCLQNILGVILFLRLTWIVGVAGVLESFLIVAMCCTCTMLTAISMSAIAT
mKCC4           RMGTFIGVYLPCLQNILGVILFLRLTWIVGAAGVMESFXIVAMCCTCTMLTAISMSAIAT
hKCC2           RMGTFMGVYLPCLQNIFGVILFLRXTWVVGIAGIMESFCMVFICCSCTMLTAISMSAIAT
                :****:**********:******* **:** **::::* :* :** **************

mKCC3a-X2       NGVVPAGGSYFMISRALGPEFGGAVGLCFYLGTTFAAAMYILGAIEIFLVYIVPRAAIFR
mKCC3a          NGVVPAGGSYFMISRALGPEFGGAVGLCFYLGTTFAAAMYILGAIEIFLVYIVPRAAIFR
hKCC3a-X2       NGVVPAGGSYFMISRALGPEFGGAVGLCFYLGTTFAAAMYILGAIEIFLVYIVPRAAIFH
hKCC3a          NGVVPAGGSYFMISRALGPEFGGAVGLCFYLGTTFAAAMYILGAIEIFLVYIVPRAAIFH
mKCC3b          NGVVPAGGSYFMISRALGPEFGGAVGLCFYLGTTFAAAMYILGAIEIFLVYIVPRAAIFR
hKCC4           NGVVPAGGSYYMISRSLGPEFGGAVGLCFYLGTTFAGAMYILGTIEIFLTYISPGAAIFQ
mKCC4           NGVVPAGGSYYMISRSLGPEFGGAVGLCFYLGTTFAGAMYILGTIEIFLTYISPSAAIFQ
hKCC2           NGVVPAGGSYYMISRSLGPEFGGAVGLCFYLGTTFAGAMYILGTIEILLAYLFPAMAIFK
                **********:****:********************.******:***:*.*: *  ***:

mKCC3a-X2       SDDALKESAAMLNNMRVYGTAFLVLMVLVVFIGVRYVNKFASLFLACVIVSILAIYAGAI
mKCC3a          SDDALKESAAMLNNMRVYGTAFLVLMVLVVFIGVRYVNKFASLFLACVIVSILAIYAGAI
hKCC3a-X2       SDDALKESAAMLNNMRVYGTAFLVLMVLVVFIGVRYVNKFASLFLACVIVSILAIYAGAI
hKCC3a          SDDALKESAAMLNNMRVYGTAFLVLMVLVVFIGVRYVNKFASXFLACVIVSILAIYAGAI
mKCC3b          SDDALKESAAMLNNMRVYGTAFLVLMVLVVFIGVRYVNKFASLFLACVIVSILAIYAGAI
hKCC4           AEAAGGEAAAMLHNMRVYGTCTLVLMALVVFVGVKYVNKLALVFLACVVLSILAIYAGVI
mKCC4           AETADGEAAALLNNMRVYGSCALALMAVVVFVGVKYVNKLALVFLACVVLSILAIYAGVI
hKCC2           AEDASGEAAAMLNNMRVYGTCVLTCMATVVFVGVKYVNKFALVFLGCVILSILAIYAGVI
                :: *  *:**:*:******:. *. *. ***:**:****:*  **.**::********.*
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mKCC3a-X2       KSSFAPPHFPVCMLGNRTLSSRHLDICSKTKEVDNMTVPSKLWGFFCNSSQFFNATCDEY
mKCC3a          KSSFAPPHFPVCMLGNRTLSSRHLDICSKTKEVDNMTVPSKLWGFFCNSSQFFNATCDEY
hKCC3a-X2       KSSFAPPHFPVCMLGNRTLSSRHIDVCSKTKEINNMTVPSKLWGFFCNSSQFFNATCDEY
hKCC3a          KSSFAPPHFPVCMLGNRTLSSRHIDVCSKTKEINNMTVPSKLWGFFCNSSQFFNATCDEY
mKCC3b          KSSFAPPHFPVCMLGNRTLSSRHLDICSKTKEVDNMTVPSKLWGFFCNSSQFFNATCDEY
hKCC4           KSAFDPPDIPVCLLGNRTLSRRSFDACVKAYGIHNNSATSALWGLFCNGS-QPSAACDEY
mKCC4           KTAFAPPDIPVCLLGNRTLANRNFDTCAKMQVVSNGTVTTALWRLFCNGS-SLGATCDEY
hKCC2           KSAFDPPNFPICLLGNRTLSRHGFDVCAKLAWEGNETVTTRLWGLFCSSR-FLNATCDEY
                *::* **.:*:*:******: : :* * *     * :..: ** :**..    .*:****

mKCC3a-X2       FVHNNVISIQGIPGLASGIITENLWSNYLPKGEIIEKPSAKSSDVLG----NLNHEYVLA
mKCC3a          FVHNNVISIQGIPGLASGIITENLWSNYLPKGEIIEKPSAKSSDVLG----NLNHEYVLA
hKCC3a-X2       FVHNNVTSIQGIPGLASGIITENLWSNYLPKGEIIEKPSAKSSDVLG----SLNHEYVLV
hKCC3a          FVHNNVTSIQGIPGLASGIITENLWSNYLPKGEIIEKPSAKSSDVLG----SLNHEYVLV
mKCC3b          FVHNNVISIQGIPGLASGIITENLWSNYLPKGEIIEKPSAKSSDVLG----NLNHEYVLA
hKCC4           FIQNNVTEIQGIPGAASGVFLENLWSTYAHAGAFVEKKGVPSVPVAE-ESRASTLPYVLT
mKCC4           FAQNNVTEIQGIPGVASGVFLDNLWSTYSDKGAFVEKKGVSSVPVSE-ESRPGGLPYVLT
hKCC2           FTRNNVTEIQGIPGAASGLIKENXWSSYLTKGVIVERSGMTSVGLADGTPIDMDHPYVFS
                * :*** .****** ***:: :* **.*   * ::*: .  *  :          **:

mKCC3a-X2       DITTSFTLLVGIFFPSVTGIMAGSNRSGDLKDAQKSIPIGTILAILTTSFVYLSNVVLFG
mKCC3a          DITTSFTLLVGIFFPSVTGIMAGSNRSGDLKDAQKSIPIGTILAILTTSFVYLSNVVLFG
hKCC3a-X2       DITTSFTXLVGIFFPSVTGIMAGSNRSGDLKDAQKSIPIGTILAILTTSFVYLSNVVLFG
hKCC3a          DITTSFTLLVGIFFPSVTGIMAGSNRSGDLKDAQKSIPIGTILAILTTSFVYLSNVVLFG
mKCC3b          DITTSFTLLVGIFFPSVTGIMAGSNRSGDLKDAQKSIPIGTILAILTTSFVYLSNVVLFG
hKCC4           DIAASFTLLVGIYFPSVTGIMAGSNRSGDLKDAQKSIPTGTILAIVTTSFIYLSCIVLFG
mKCC4           DIMTYFTMLVGIYFPSVTGIMAGSNRSGDLKDAQKSIPTGTILAIVTTSFIYXSCIVLFG
hKCC2           DMTSYFTLLVGIYFPSVTGIMAGSNRSGDLRDAQKSIPTGTILAIATTSAVYISSVVLFG
                *: : ** ****:*****************:******* ****** *** :* * :****

mKCC3a-X2       ACIEGVVLRDKFGDAVKGNLVVGTLSWPSPWVIVIGSFFSTCGAGLQSLTGAPRLLQAIA
mKCC3a          ACIEGVVLRDKFGDAVKGNLVVGTLSWPSPWVIVIGSFFSTCGAGLQSLTGAPRLLQAIA
hKCC3a-X2       ACIEGVVLRDKFGDAVKGNLVVGTLSWPSPWVIVIGSFFSTCGAGLQSLTGAPRLLQAIA
hKCC3a          ACIEGVVLRDKFGDAVKGNLVVGTLSWPSPWVIVIGSFFSTCGAGLQSLTGAPRLLQAIA
mKCC3b          ACIEGVVLRDKFGDAVKGNLVVGTLSWPSPWVIVIGSFFSTCGAGLQSXTGAPRLLQAIA
hKCC4           ACIEGVVLRDKFGEALQGNLVIGMLAWPSPWVIVIGSFFSTCGAGLQTLTGAPRLLQAIA
mKCC4           ACIEGVVLRDKFGEALQGNLVIGMLAWPSPWVIVIGSFFSTCGAGLQSLTGAPRLLQAIA
hKCC2           ACIEGVVLRDKFGEAVNGNLVVGTLAWPSPWVIVIGSFFSTCGAGLQSLTGAPRLLQAIS
                *************:*::****:* *:**********************: **********:

mKCC3a-X2       KDNIIPFLRVFGHSKANGEPTWALLLTAAIAELGILIASLDLVAPIXSMFFLMCYLFVNL
mKCC3a          KDNIIPFLRVFGHSKANGEPTWALLLTAAIAELGILIASLDLVAPILSMFFLMCYLFVNL
hKCC3a-X2       KDNIIPFLRVFGHSKANGEPTWALLLTAAIAELGILIASLDLVAPILSMFFLMCYLFVNL
hKCC3a          KDNIIPFLRVFGHSKANGEPTWALLLTAAIAELGILIASLDLVAPILSMFFLMCYLFVNL
mKCC3b          KDNIIPFLRVFGHSKANGEPTWALLLTAAIAELGILIASLDLVAPILSMFFLMCYLFVNL
hKCC4           RDGIVPFLQVFGHGKANGEPTWALLLTVLICETGILIASLDSVAPILSMFFLMCYLFVNL
mKCC4           RDGIIPFLQVFGHGKANGEPTWALLLTALICETGILIASLDSVAPILSMFFXMCYMFVNL
hKCC2           RDGIVPFLQVFGHGKANGEPTWALLLTACICEIGILIASLDEVAPILSMFFLMCYMFVNL
                :*.*:***:****.*************. *.* ******** **** **** ***:****

mKCC3a-X2       ACALQTLLRTPNWRPRFRYYHWALSFMGMSICLALMFISSWYYAIVAMVIAGMIYKYIEY
mKCC3a          ACALQTLLRTPNWRPRFRYYHWALSFMGMSICLALMFISSWYYAIVAMVIAGMIYKYIEY
hKCC3a-X2       ACALQTLLRTPNWRPRFRYYHWALSFMGMSICLALMFISSWYYAIVAMVIAGMIYKYIEY
hKCC3a          ACALQTLLRTPNWRPRFRYYHWALSFMGMSICLALMFISSWYYAIVAMVIAGMIYKYIEY
mKCC3b          ACALQTLLRTPNWRPRFRYYHWALSFMGMSICLALMFISSWYYAIVAMVIAGMIYKYIEY
hKCC4           ACAVQTLLRTPNWRPRFKFYHWTLSFLGMSLCLALMFICSWYYALSAMLIAGCIYKYIEY
mKCC4           ACAVQTLLRTPNWRPRFKFYHWTLSFLGMSLCLALMFICSWYYAXFAMLIAGCIYKYIEY
hKCC2           ACAVQTLLRTPNWRPRFRYYHWTLSFLGMSLCLALMFICSWYYALVAMLIAGLIYKYIEY
                ***:*************:::***:***:***:*******.*****  **:*** *******
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mKCC3a-X2       QGAEKEWGDGIRGLSLSAARFALLRLEEGPPHTKNWRPQLLVLLKLDEDLHVKHPRLLTF
mKCC3a          QGAEKEWGDGIRGLSLSAARFALLRLEEGPPHTKNWRPQLLVLLKLDEDLHVKHPRLLTF
hKCC3a-X2       QGAEKEWGDGIRGLSLSAARFALLRLEEGPPHTKNWRPQLLVLLKLDEDLHVKHPRLLTF
hKCC3a          QGAEKEWGDGIRGLSLSAARFALLRLEEGPPHTKNWRPQLLVLLKLDEDLHVKHPRLLTF
mKCC3b          QGAEKEWGDGIRGLSLSAARFALLRLEEGPPHTKNWRPQLLVLLKLDEDLHVKHPRLLTF
hKCC4           RGAEKEWGDGIRGLSLNAARYALLRVEHGPPHTKNWRPQVLVMLNLDAEQAVKHPRLLSF
mKCC4           RGAEKEWGDGIRGLSLNAARYALLRVEHGPPHTKNWRPQVLVMLNLDSEQCVKHPRLLSF
hKCC2           RGAEKEWGDGIRGLSLSAARYALLRLEEGPPHTKNWRPQLLVLVRVDQDQNVVHPQLLSL
                :***************.***:****:*.***********:**:::.:* :  * **:**::

mKCC3a-X2       ASQLKAGKGLTIVGSVIVGNFLENYG---DAEQTIKHLMEAEKVKGFCQLVVAAKLKEGI
mKCC3a          ASQLKAGKGLTIVGSVIVGNFLENYG---DAEQTIKHLMEAEKVKGFCQLVVAAKLKEGI
hKCC3a-X2       ASQLKAGKGLTIVGSVIVGNFLENYG---EAEQTIKHLMEAEKVKGFCQLVVAAKLREGI
hKCC3a          ASQLKAGKGXTIVGSVIVGNFLENYG---EAEQTIKHLMEAEKVKGFCQLVVAAKLREGI
mKCC3b          ASQLKAGKGLTIVGSVIVGNFLENYG---DAEQTIKHLMEAEKVKGFCQLVVAAKLKEGI
hKCC4           TSQLKAGKGLTIVGSVLEGTYLDKHMEAQRAEENIRSLMSTEKTKGFCQLVVSSSLRDGM
mKCC4           TSQLKAGKGLTIVGSVLEGTYLDKHVEAQRAEENIRSLMSAEKTKGFCQLVVSSNLRDGA
hKCC2           TSQLKAGKGLTIVGSVLEGTFLENHPQAQRAEESIRRLMEAEKVKGFCQVVISSNLRDGV
                :******** ******: *.:*:::     **:.*: **.:**.*****:*::::.*::*

mKCC3a-X2       SHLIQSCGLGGMKHNTVVMGWPNGWRQSEDARAWKTFIGTVRVTTAAHLALLVAKNVSFF
mKCC3a          SHLIQSCGLGGMKHNTVVMGWPNGWRQSEDARAWKTFIGTVRVTTAAHLALLVAKNVSFF
hKCC3a-X2       SHLIQSCGLGGMKHNTVVMGWPNGWRQSEDARAWKTFIGTVRVTTAAHLALLVAKNISFF
hKCC3a          SHLIQSCGLGGMKHNTVVMGWPNGWRQSEDARAWKTFIGTVRVTTAAHLALLVAKNISFF
mKCC3b          SHLIQSCGLGGMKHNTVVMGWPNGWRQSEDARAWKTFIGTVRVTTAAHLALLVAKNVSFF
hKCC4           SHLIQSAGLGGLKHNTVLMAWPASWKQEDNPFSWKNFVDTVRDTTAAHQALLVAKNVDSF
mKCC4           SHLIQSAGLGGMKHNTVLMAWPEAWKEADNPFSWKNFVDTVRDTTAAHQALLVAKNIDLF
hKCC2           SHLIQSGGLGGLQHNTVLVGWPRNWRQKEDHQTWRNFIELVRETTAGHLALLVTKNVSMF
                ****** ****::****:.** *:: ::  :*:.*:  ** ***.* ****:**:. *

mKCC3a-X2       PSNVEQFSEGNIDVRWIVHDGGMLMLLPFLLKQHKVWRKCSIRIFTVAQLEDNSIQMKKD
mKCC3a          PSNVEQFSEGNIDVRWIVHDGGMLMLLPFLLKQHKVWRKCSIRIFTVAQLEDNSIQMKKD
hKCC3a-X2       PSNVEQFSEGNIDVWWIVHDGGMLMLLPFLLKQHKVWRKCSIRIFTVAQLEDNSIQMKKD
hKCC3a          PSNVEQFSEGNIDVWWIVHDGGMLMLLPFLLKQHKVWRKCSIRIFTVAQLEDNSIQMKKD
mKCC3b          PSNVEQFSEGNIDVRWIVHDGGMLMLLPFLLKQHKVWRKCSIRIFTVAQLEDNSIQMKKD
hKCC4           PQNQERFGGGHIDVWWIVHDGGMLMLLPFLLRQHKVWRKCRMRIFTVAQVDDNSIQMKKD
mKCC4           PQNQERFSDGNIDVWWIVHDGGMLMLLPFLLRQHKVWRKCRMRIFTVAQVDDNSIQMKKD
hKCC2           PGNPERFSEGSIDVWWIVHDGGMLMLLPFLLRHHKVWRKCKMRIFTVAQMDDNSIQMKKD
                * * *:*. * *** ****************::******* :*******::**********

mKCC3a-X2       LATFLYHLRIEAEVEVVEMHDSDISAYTYERTLMMEQRSQMLRHMRLSKTERDR------
mKCC3a          LATFLYHLRIEAEVEVVEMHDSDISAYTYERTLMMEQRSQMLRHMRLSKTERDR------
hKCC3a-X2       LATFLYHLRIEAEVEVVEMHDSDISAYTYERTLMMEQRSQMLRHMRLSKTERDR------
hKCC3a          LATFLYHLRIEAEVEVVEMHDSDISAYTYERTLMMEQRSQMLRHMRLSKTERDR------
mKCC3b          LATFLYHLRIEAEVEVVEMHDSDISAYTYERTLMMEQRSQMLRHMRLSKTERDR------
hKCC4           LQMFLYHLRISAEVEVVEMVENDISAFTYERTLMMEQRSQMLKQMQLSKNEQER------
mKCC4           LQMFLYHLRISAEVEVVEMVENDISAFTYEKTLMMEQRSQMLKQMQLSKNERER------
hKCC2           LTTFLYHLRITAEVEVVEMHESDISAYTYEKTLVMEQRSQIXKQMHLTKNERE REIQSIT
                *  ******* ******** :.****:***:**:******: ::*:*:*.*::*

mKCC3a-X2       ------------------------------------EAQLVKDRNSMLRLTSIGSDEDEE-
mKCC3a          ------------------------------------EAQLVKDRNSMLRLTSIGSDEDEE-
hKCC3a-X2       ------------------------------------EAQLVKDRNSMLRLTSIGSDEDEE-
hKCC3a          ------------------------------------EAQLVKDRNSMLRLTSIGSDEDEE-
mKCC3b          ------------------------------------EAQLVKDRNSMXRLTSIGSDEDEE-
hKCC4           ------------------------------------EAQLIHDRNTASHTAAAARTQAPP-
mKCC4           ------------------------------------EAQLIHDRNTASHTTATARTQAPP-
hKCC2           DESRGSIRRKNPANTRLRLNVPEETAGDSEEKPEEEVQLIHDQSAPSCPSSSPSPGEEPE
                                                    *.**::*:.:    ::
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mKCC3a-X2       --TETYQEKVHMTWTKDKYMAS--RGQKVKSMEGFQDLLNMRPD-----QSNVRRMHTAV
mKCC3a          --TETYQEKVHMTWTKDKYMAS--RGQKVKSMEGFQDLLNMRPD-----QSNVRRMHTAV
hKCC3a-X2       --TETYQEKVHMTWTKDKYMAS--RGQKAKSMEGFQDLLNMRPD-----QSNVRRMHTAV
hKCC3a          --TETYQEKVHMTWTKDKYMAS--RGQKAKSMEGFQDLLNMRPD-----QSNVRRMHTAV
mKCC3b          --TETYQEKVHMTWTKDKYMAS--RGQKVKSMEGFQDLLNMRPD-----QSNVRRMHTAV
hKCC4           -----TPDKVQMTWTREKLIAEKYRSRD-TSLSGFKDLFSMKPD-----QSNVRRMHTAV
mKCC4           -----TPDKVQMTWTKEKLIAEKHRNKD-TGPSGFKDLFSLKPD-----QSNVRRMHTAV
hKCC2           GEGETDPEKVHLTWTKDKSVAEKNKGPSPVSSEGIKDFFSMKPEWENLNQSNVRRMHTAV
                       :**::***::* :*.  :. .  . .*::*::.::*:     ***********

mKCC3a-X2       KLNEVIVNKSHEAKLVLLNMPGPPRNPEGDENYMEFLEVXTEGLERVLLVRGGGSEVITIYS
mKCC3a          KLNEVIVNKSHEAKLVLLNMPGPPRNPEGDENYMEFLEVLTEGLERVLLVRGGGSEVITIYS
hKCC3a-X2       KXNEVIVNKSHEAKLVLLNMPGPPRNPEGDENYMEFLEVLTEGLERVLLVRGGGSEVITIYS
hKCC3a          KLNEVIVNKSHEAKLVLLNMPGPPRNPEGDENYMEFLEVLTEGLERVLLVRGGGSEVITIYS
mKCC3b          KLNEVIVNKSHEAKLVLLNMPGPPRNPEGDENYMEFLEVLTEGLERVLLVRGGGSEVITIYS
hKCC4           KLNGVVLNKSQDAQLVLLNMPGPPKNRQGDENYMEFLEVLTEGLNRVLLVRGGGREVITIYS
mKCC4           KXNGVVLNKSQDAQLVLLNMPGPPKSRQGDENYMEFLEVLTEGLNRVLLVRGGGREVITIYS
hKCC2           RLNEVIVKKSRDAKLVLLNMPGPPRNRNGDENYMEFLEVLTEHLDRVMLVRGGGREVITIYS
                : * *:::**::*:**********:. :********** ** *:**:****** *******
```